Figure 1 Overview

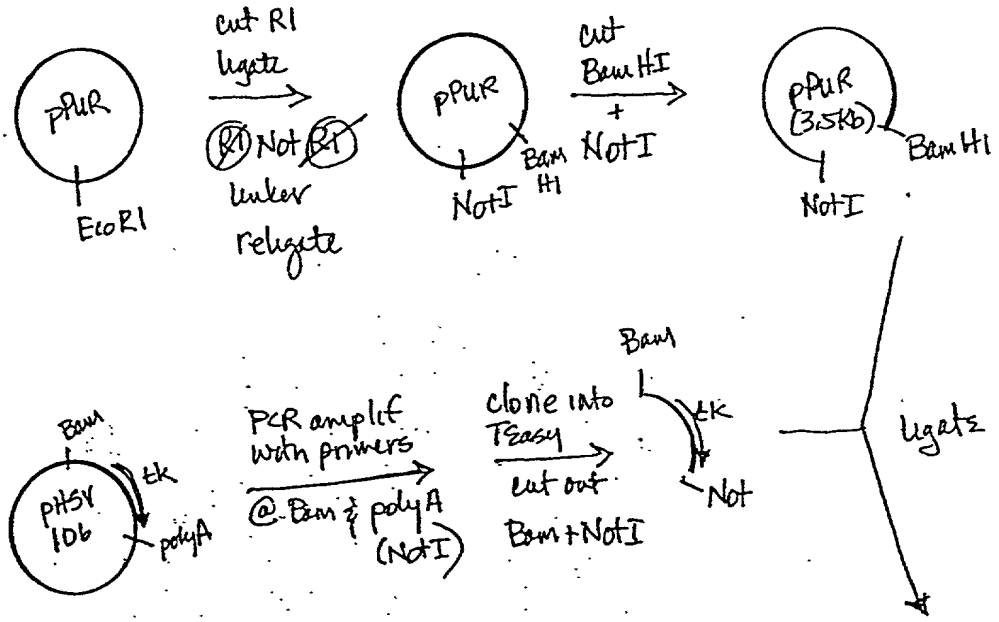
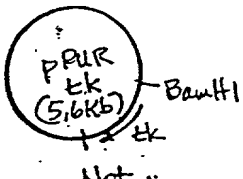
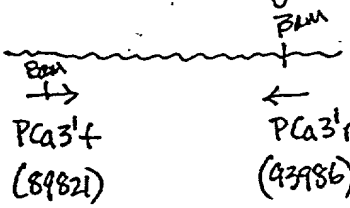


Figure 1a



4.1 Kb Bam fragment (Cα3') locus (MUSTCRA)



PCR amplify
cut with Bam

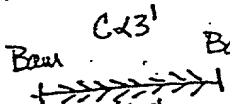


Figure 1b

Cut with Bam

ligate

screen for orientation

Figure 1c

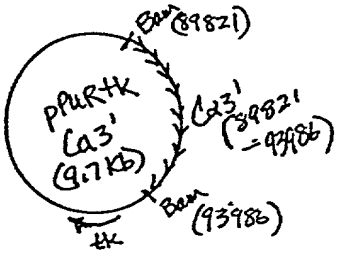


Figure 1 overview Cont.

4.8 Kb NdeI fragment (Ca5') locus MUSTCRA

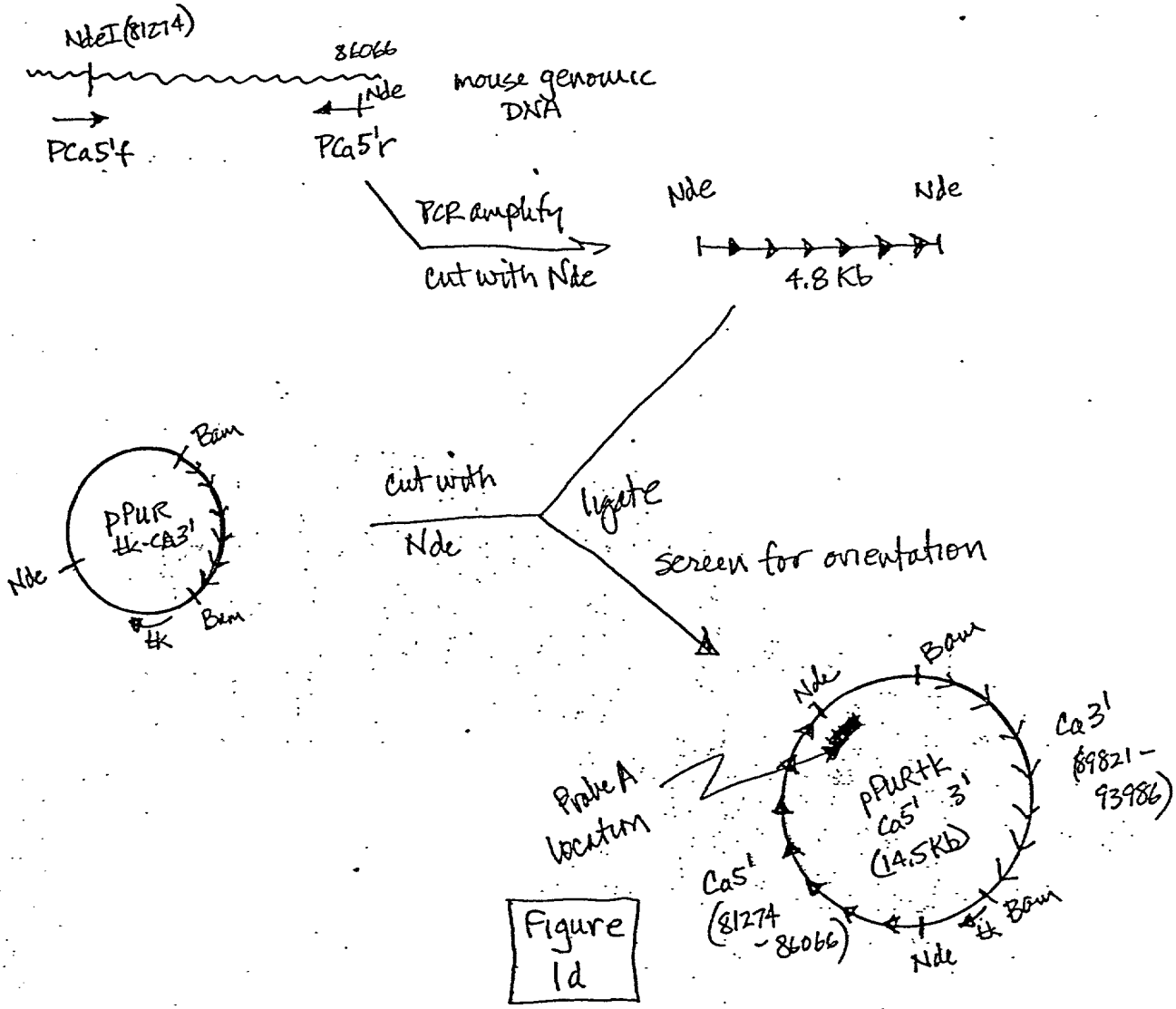


Figure 1d

T06T2T' 8h9h200T

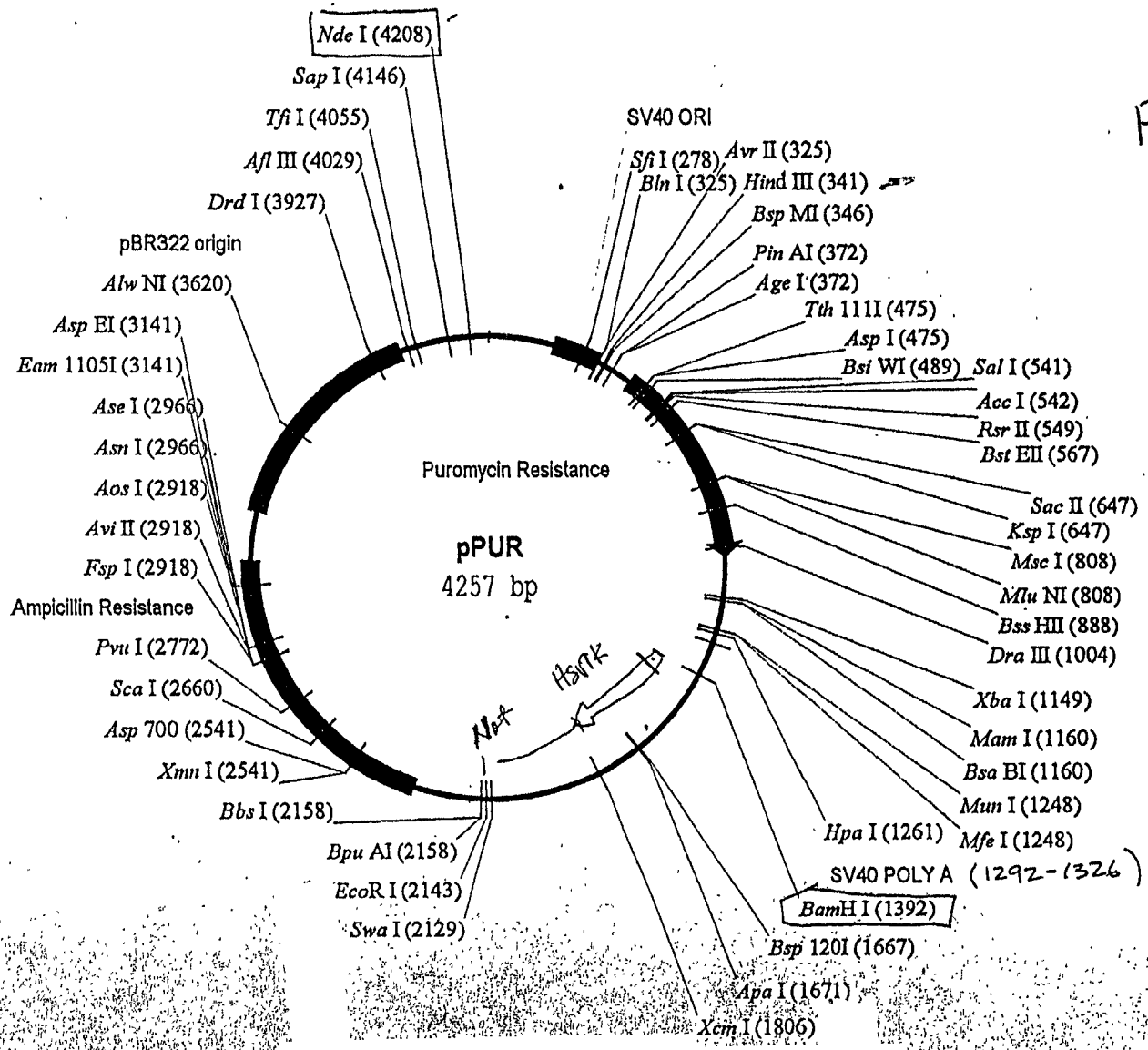
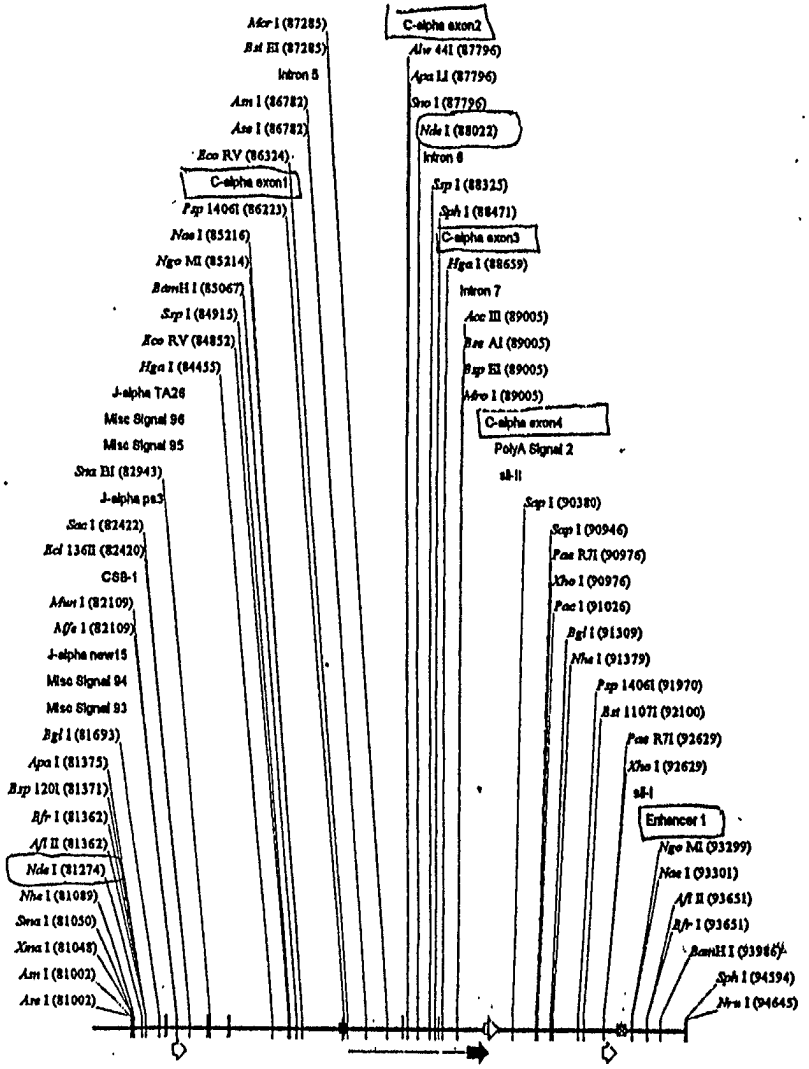


Fig. 1E

TOSTET Bhshaoot



Fragment of mTCRa,d locus (MUSTCRA)
14512 bp (molecule 94647 bp)

Fig. 1F

TOSTET 8h9h200T

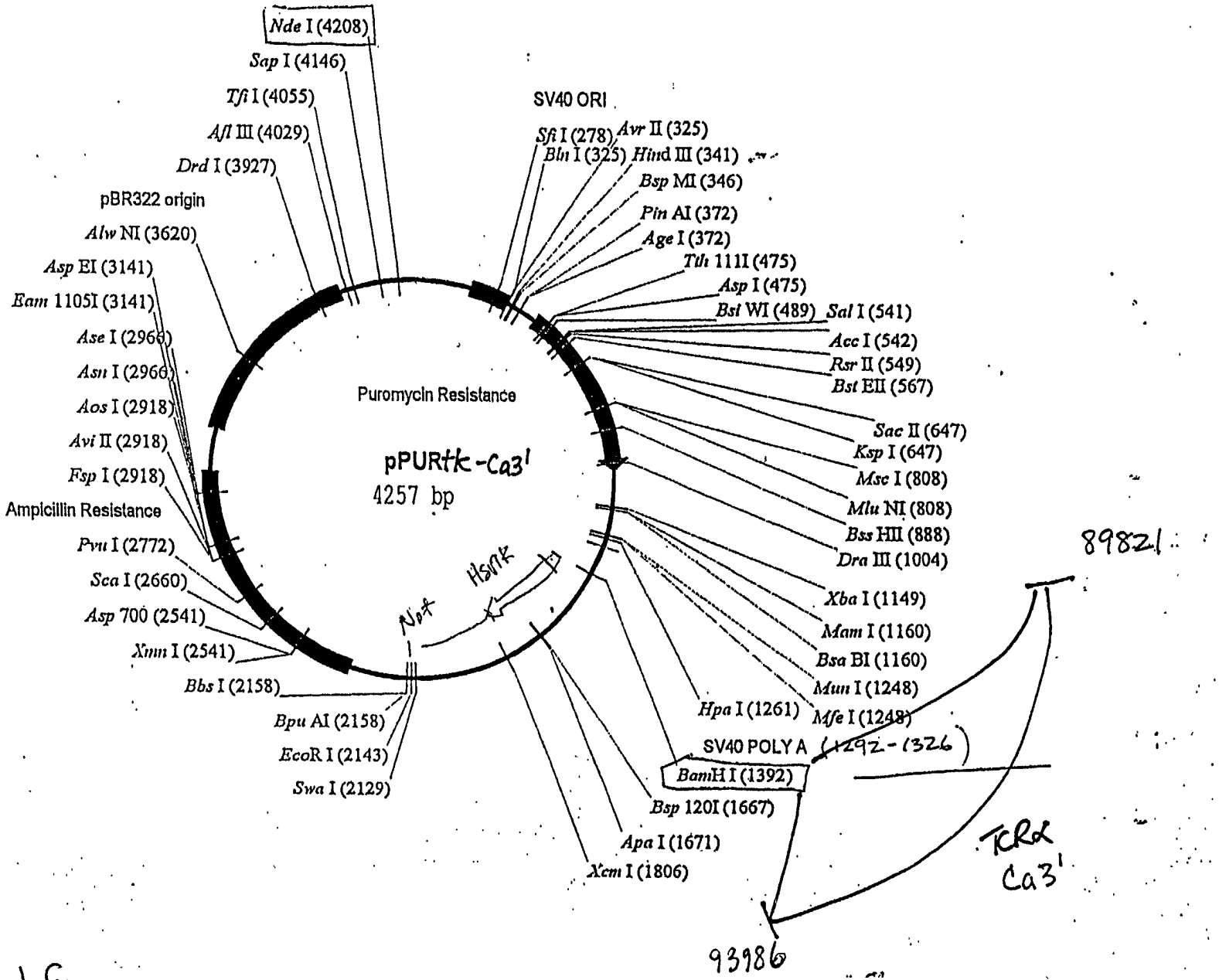


Fig. 1G

T06T2T' 8h9h200T

86066

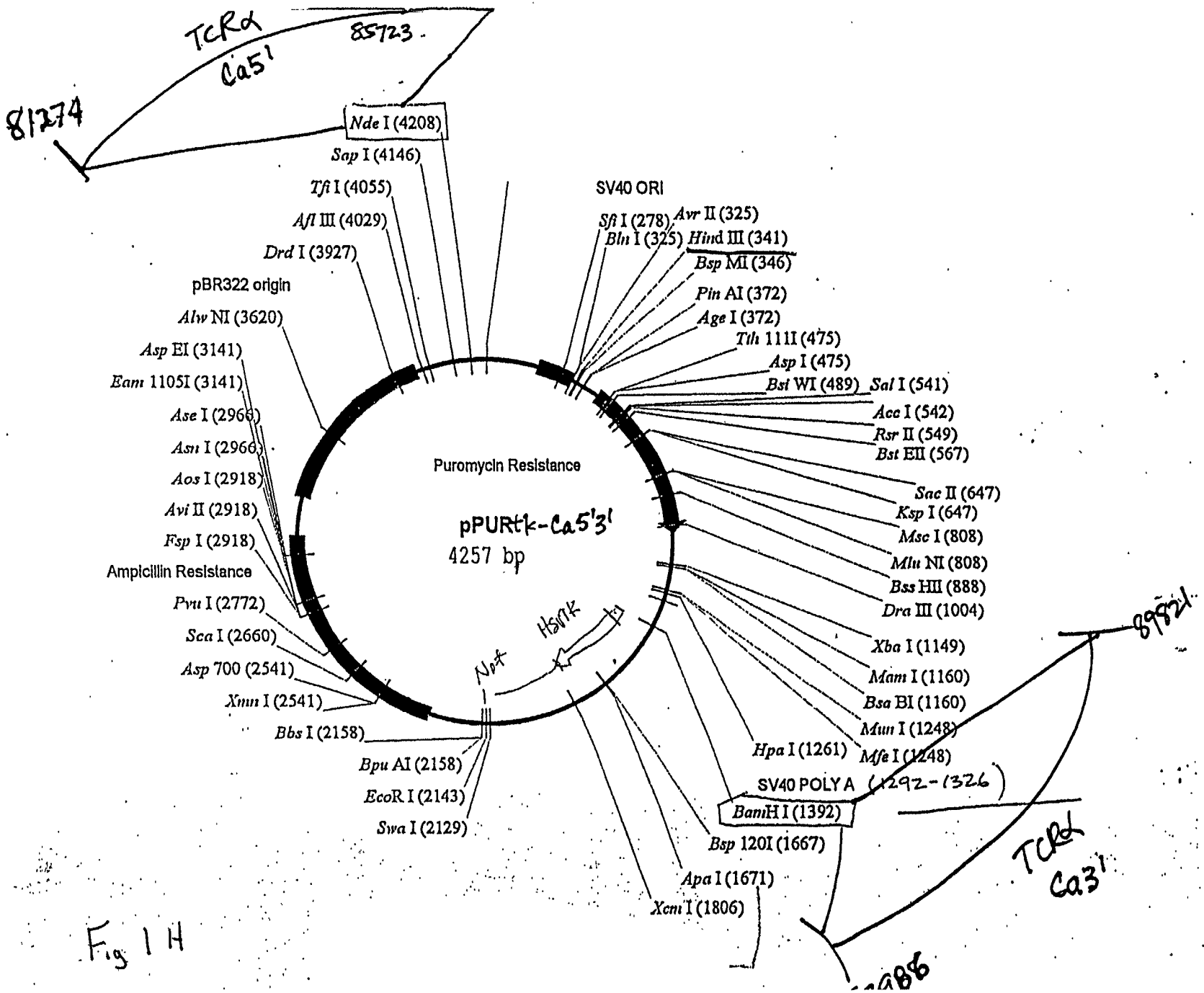
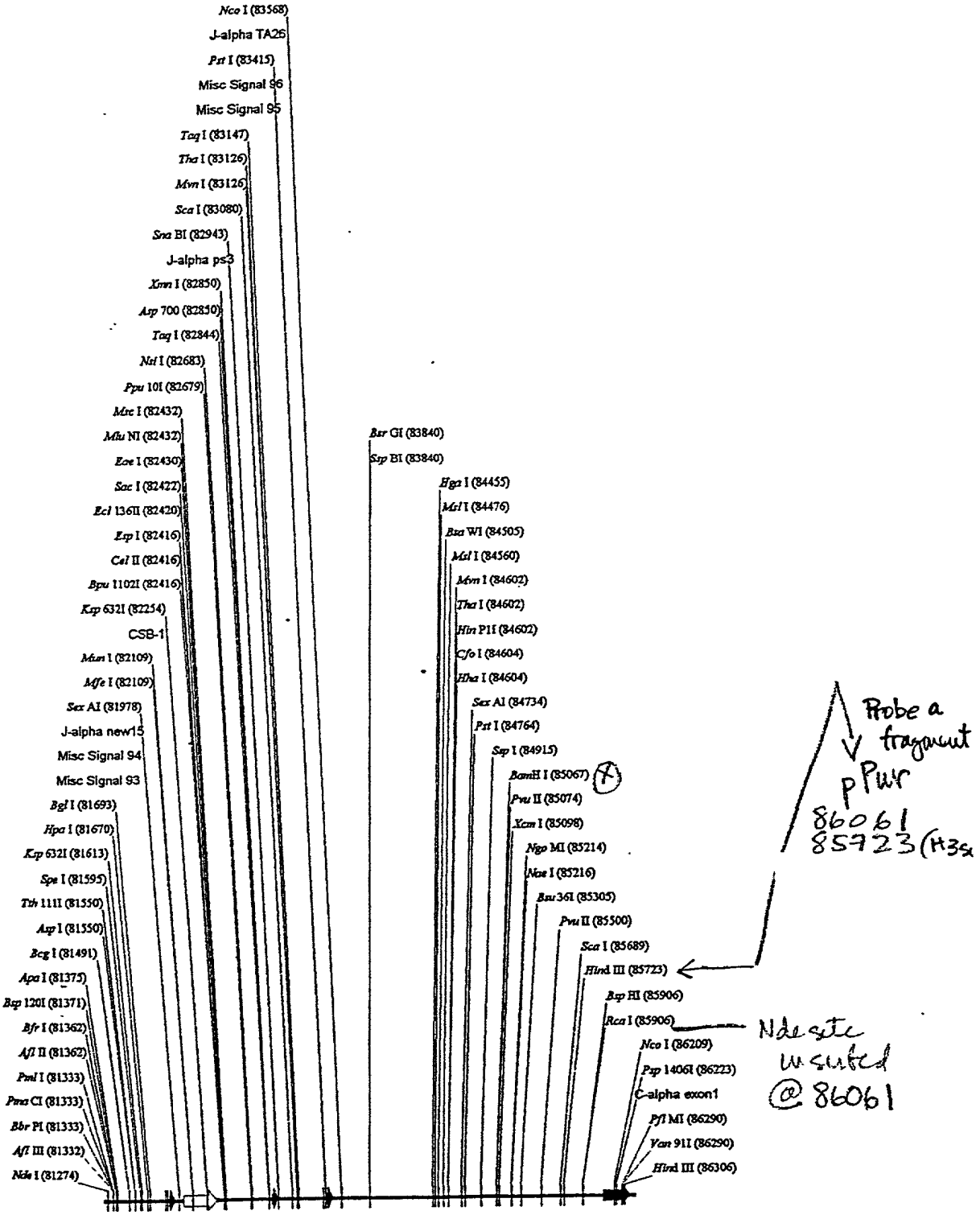


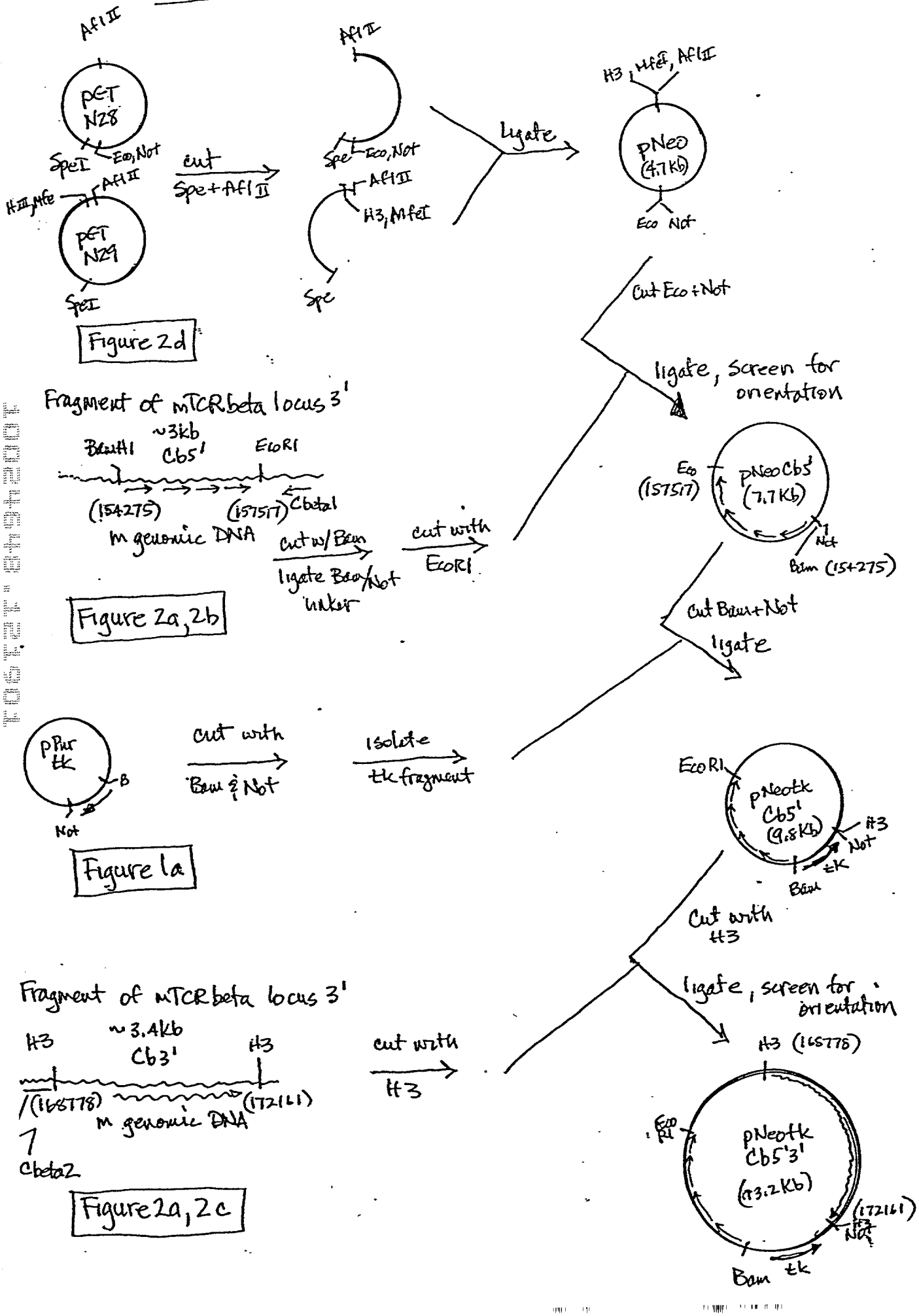
Fig 1H



Fragment of mTCRa,d locus (MUSTCRA)
5160 bp (molecule 94647 bp)

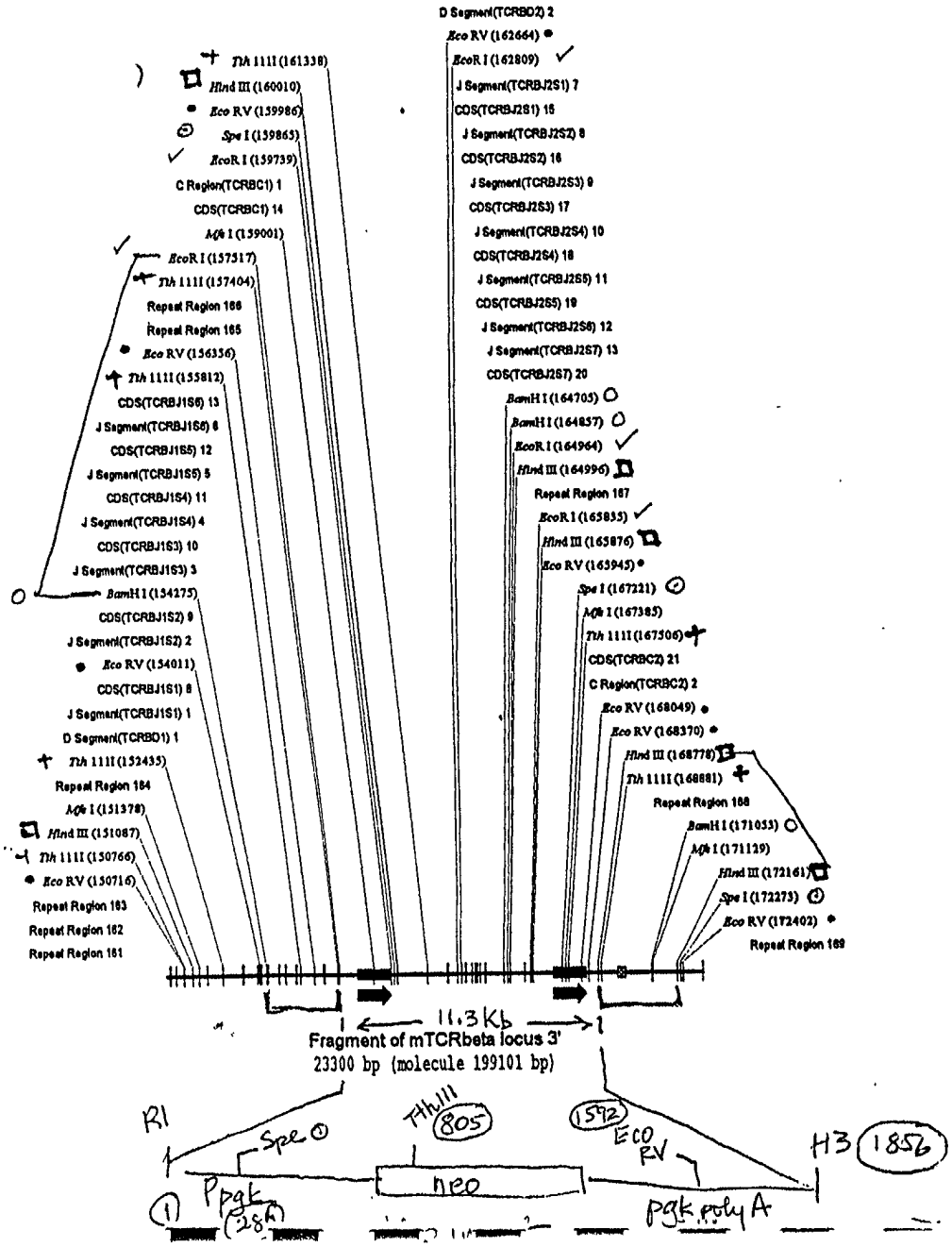
Fig. 1I

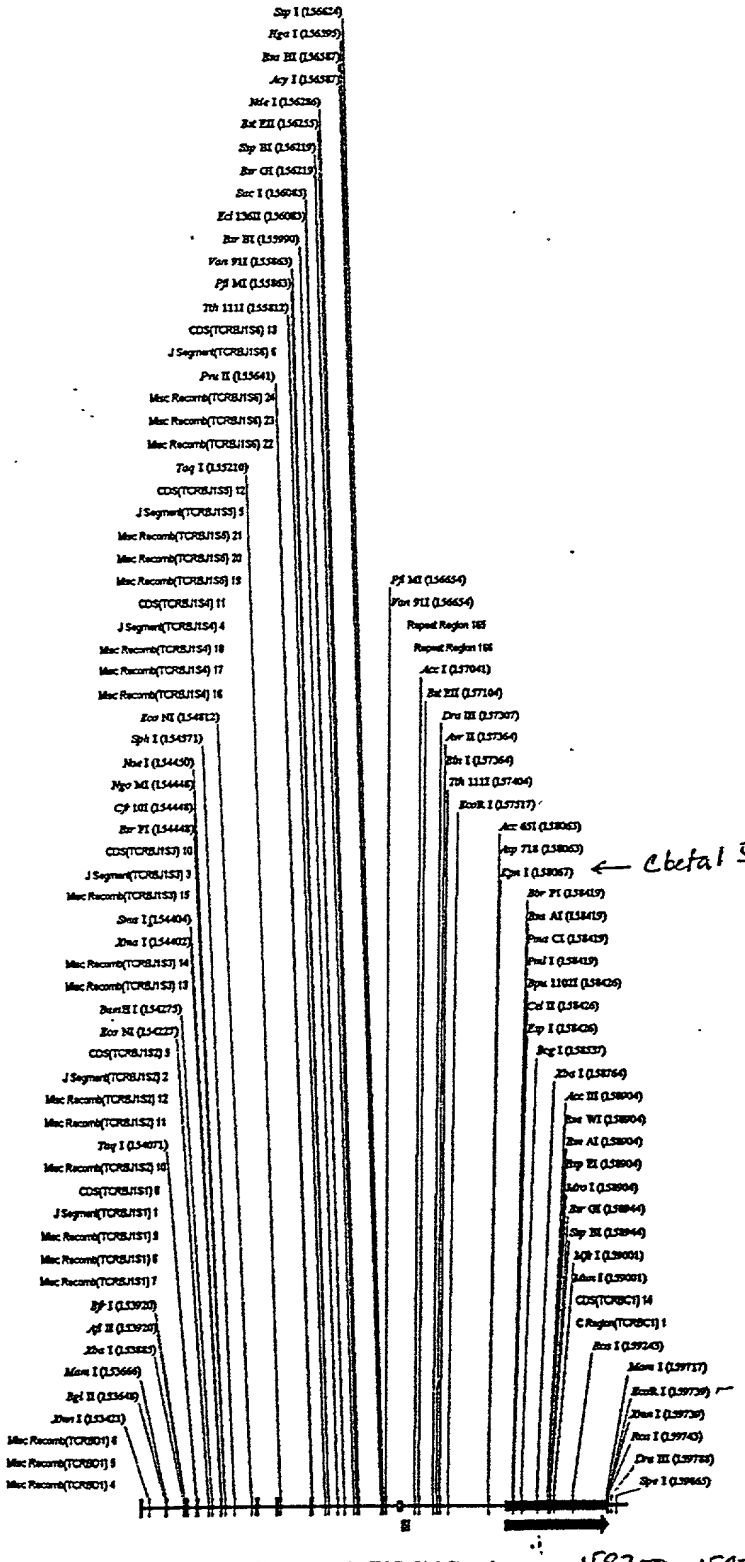
Figure 2 overview



T06T2T 8h9m200T

Fig 2a



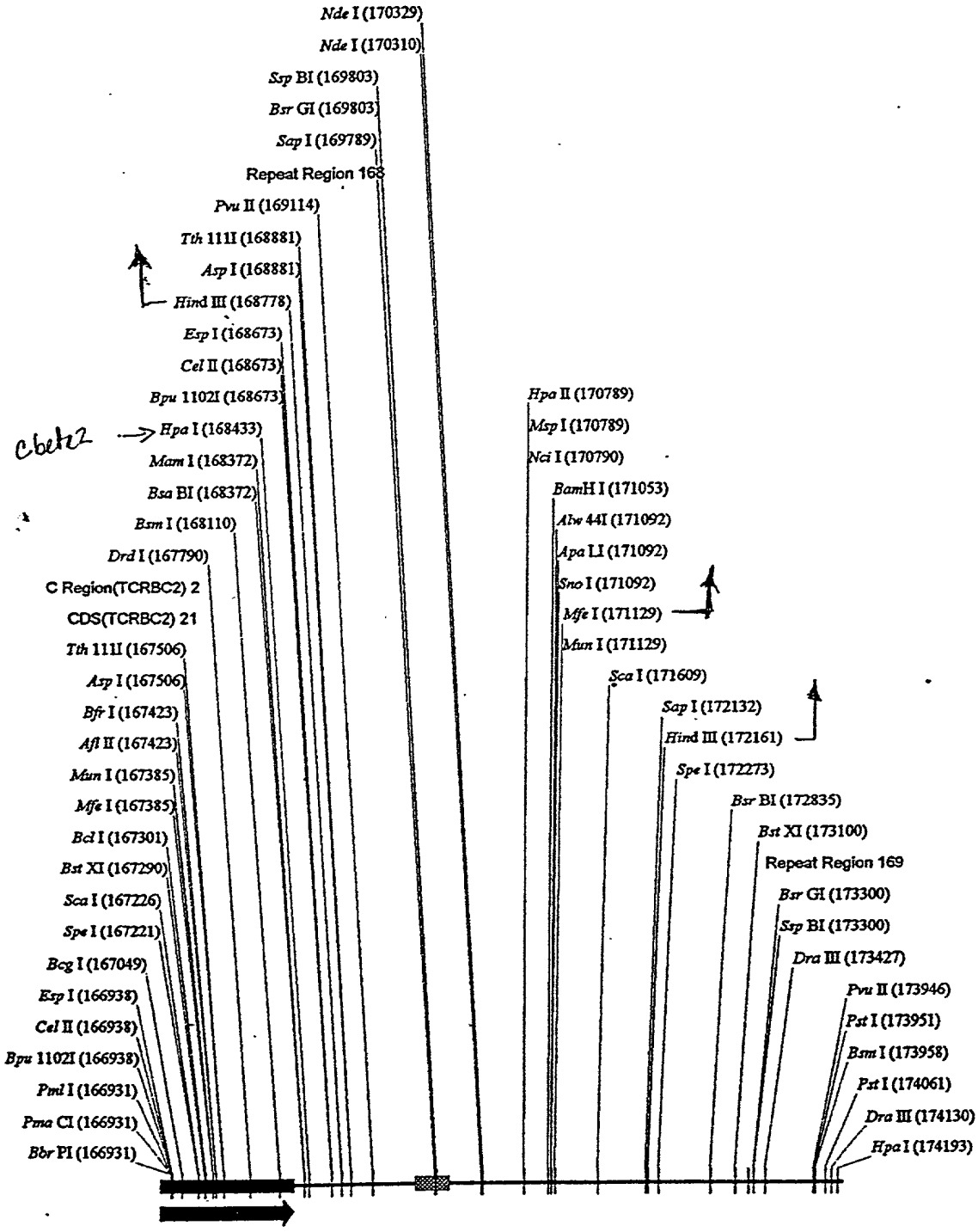


Fragment of mTCR Cb1 5' region
6700 bp (molecule 199101 bp)

← Cbeta1 5'

-158300 -159747

Fig. 2b'



Fragment of mTCRbeta locus 3'
7439 bp (molecule 199101 bp)

Fig. 2c

Construction of pNEOtk-Cb5'3'
① = pNeo
② = pNeo-Cb5'
③ = pNeotk Cb5'
④ = pNeotk-Cb5'3'
① SpeI→AflII from pGT-N39
pGT-N28
= pNEO
④ H3 1687/8 3.4Kb H3
H3 1721/6 MfeI
2.35Kb
MfeI
Afl II 4445
Acc65 I - Kpn I 4426
Sac I 4419
Apa I 4412
Aat II 4392
BamH I 4369
Hind III 4376
BsaB I 4364
PflM I 4222
EcoR V 4112
Xmn I 3948
BstX I 3937
BspD I- Cla I 3881
Rsr II 3725
Nae I - NgoM IV 3710
BsmF I 3347
Tth111 I 3325
PflF I 3325
Msc I 3290
Sfo I 3209
Kas I- Nar I 3209
PpuM I 2972
Blp I 2833
Spe I 2806
② Apa I 2539
③ EcoR I 2520
⑤ PaeR7 I- Xho I 2514
⑥ BsiW I 2507
⑪ Nsi I 2492
⑨ BstB I 2487
⑦ SnaB I 2445
⑧ Not I 2465
Afl III 2309
Hpa I 1
Bcg I 393
Ase I 754
Bsa I 858
Swa I 1099
MCS II
PGK polyA signal
M13 ori
PGK promoter
MCS I
colE1 ori
pGT-N28
pGT-N29
pGT-N38
pGT-N39
Neo^r
Not I
Nde I
Hind III
BamH I
EcoR I
Nhe I
Mlu I
BspE I
BsrG I
Sal I (Ace I)
Afl II
Nsi I
Kpn I (Acc65 I)
Sac I
Avr II
Xba I*
Aat II
Xho I
BstW I
Nde I (Ppu10 I)
BsiW I
Eco81 I
Nhe I
Xba I*
Avr II
Sac I
Kpn I (Acc65 I)
Nde I (Ppu10 I)
Bsp120 I
Pst I
Sph I
Hind III
Nco I
Xba I
Bgl II
Nhe I
Sac I
Kpn I
Sma I
Bam HI
Xho I
Eco RI
Sma I
Pst I
Sph I
Hind III
Xba I
Bam HI
Xho I
Eco RI
Sac I
Kpn I
Sma I
Bgl II
Nhe I
Sac II
Not I
Xba I
Spe I
Sal I
Pst I
Eco 47 III
Afl II
Nsi I
Nde I
Bsp120 I
Nco I
Xba

FIG 3

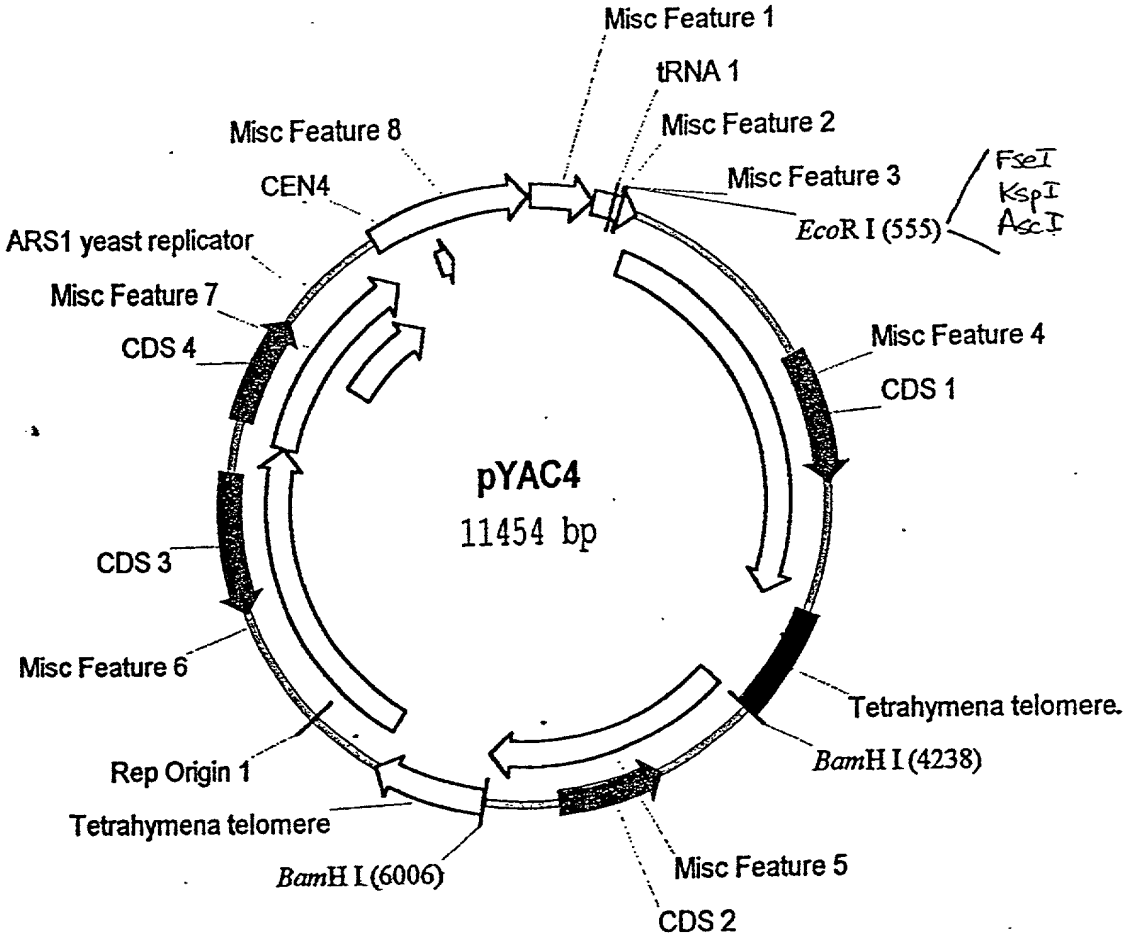
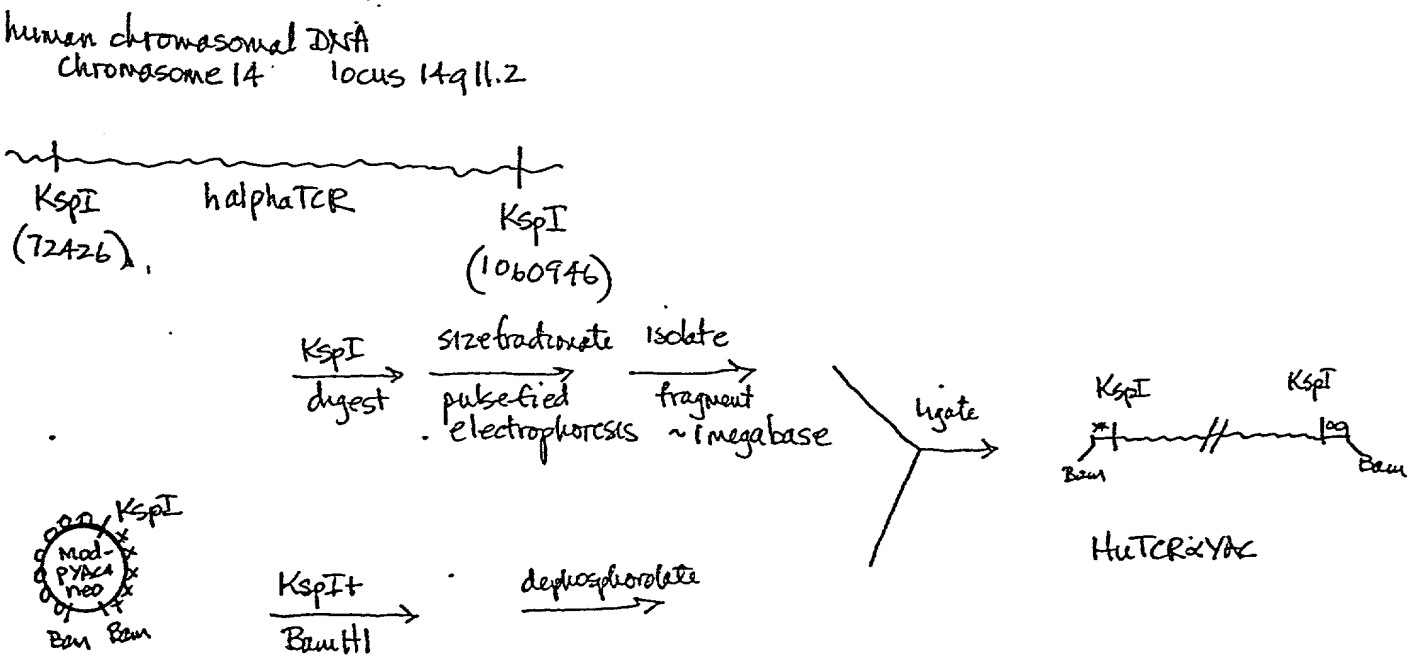

Misc Feature 1
tRNA 1
Misc Feature 2
Misc Feature 3
EcoR I (555)
FseI
KspI
AscI
Misc Feature 4
CDS 1
Tetrahymena telomere
BamH I (4238)
Misc Feature 5
CDS 2
BamH I (6006)
Tetrahymena telomere
Rep Origin 1
Misc Feature 6
CDS 3
CDS 4
Misc Feature 7
ARS1 yeast replicator
CEN4
Misc Feature 8
pYAC4
11454 bp

Figure 4. OVERVIEW



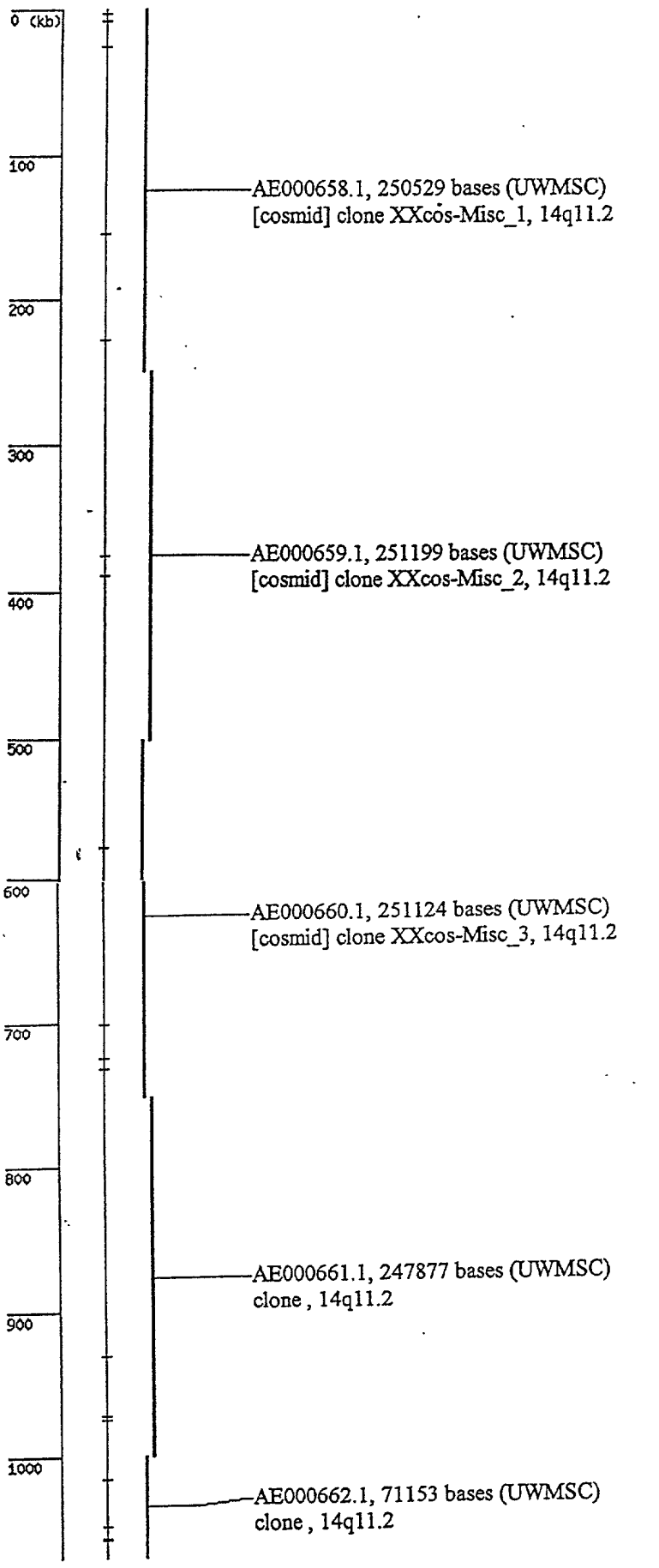
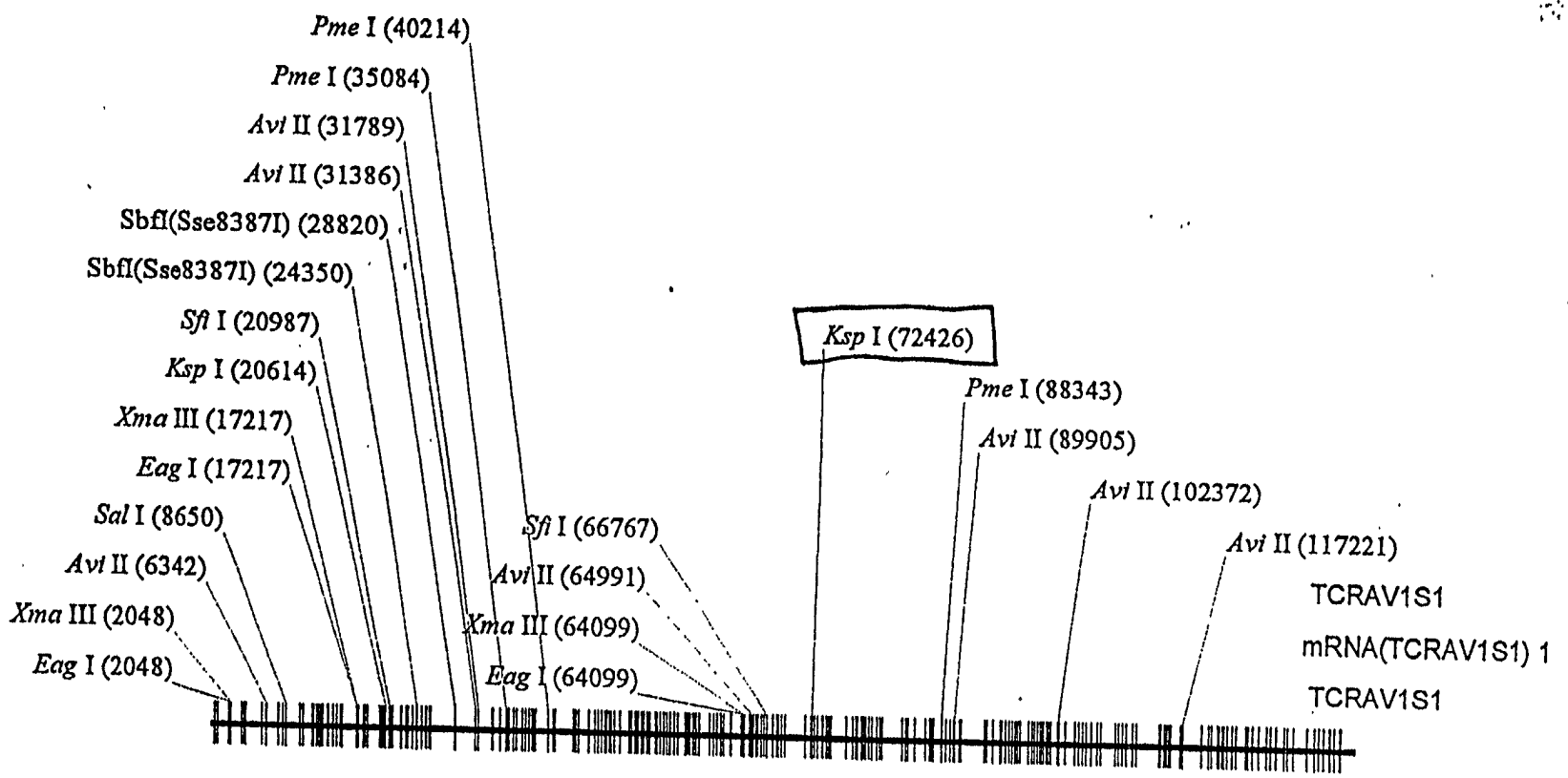

Mar
Com
0 (kb)
100
200
300
400
500
600
700
800
900
1000
AE000658.1, 250529 bases (UWMSC)
[cosmid] clone XXcos-Misc_1, 14q11.2
AE000659.1, 251199 bases (UWMSC)
[cosmid] clone XXcos-Misc_2, 14q11.2
AE000660.1, 251124 bases (UWMSC)
[cosmid] clone XXcos-Misc_3, 14q11.2
AE000661.1, 247877 bases (UWMSC)
clone , 14q11.2
AE000662.1, 71153 bases (UWMSC)
clone , 14q11.2

Fig. 4A

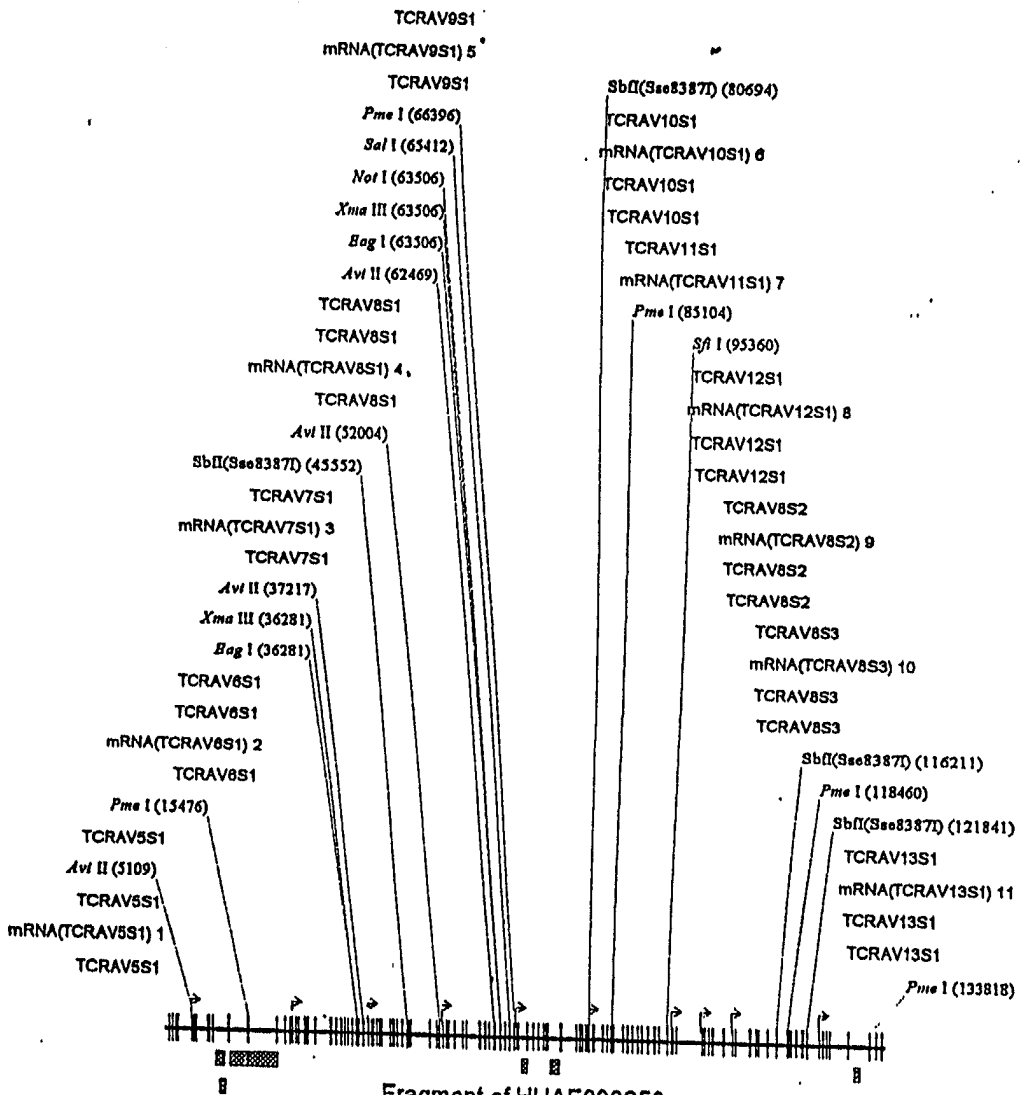
T0S737 8h9n20OF



Fragment of HUAE000658
137784 bp (molecule 250529 bp)

Fig 4b

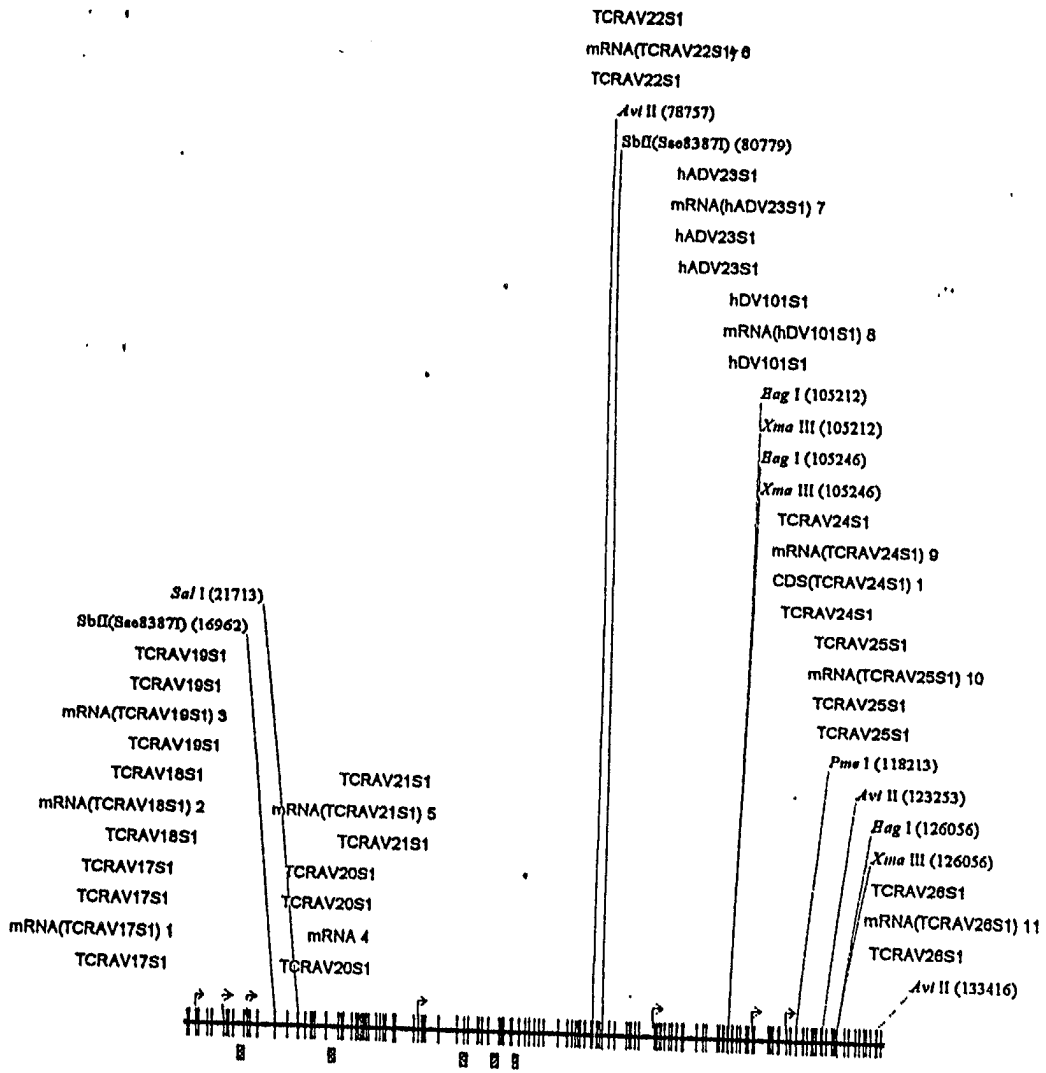
T06T2T 8h9h20OT



Fragment of HUAE000659
136312 bp (molecule 251199 bp)

Fig 4C

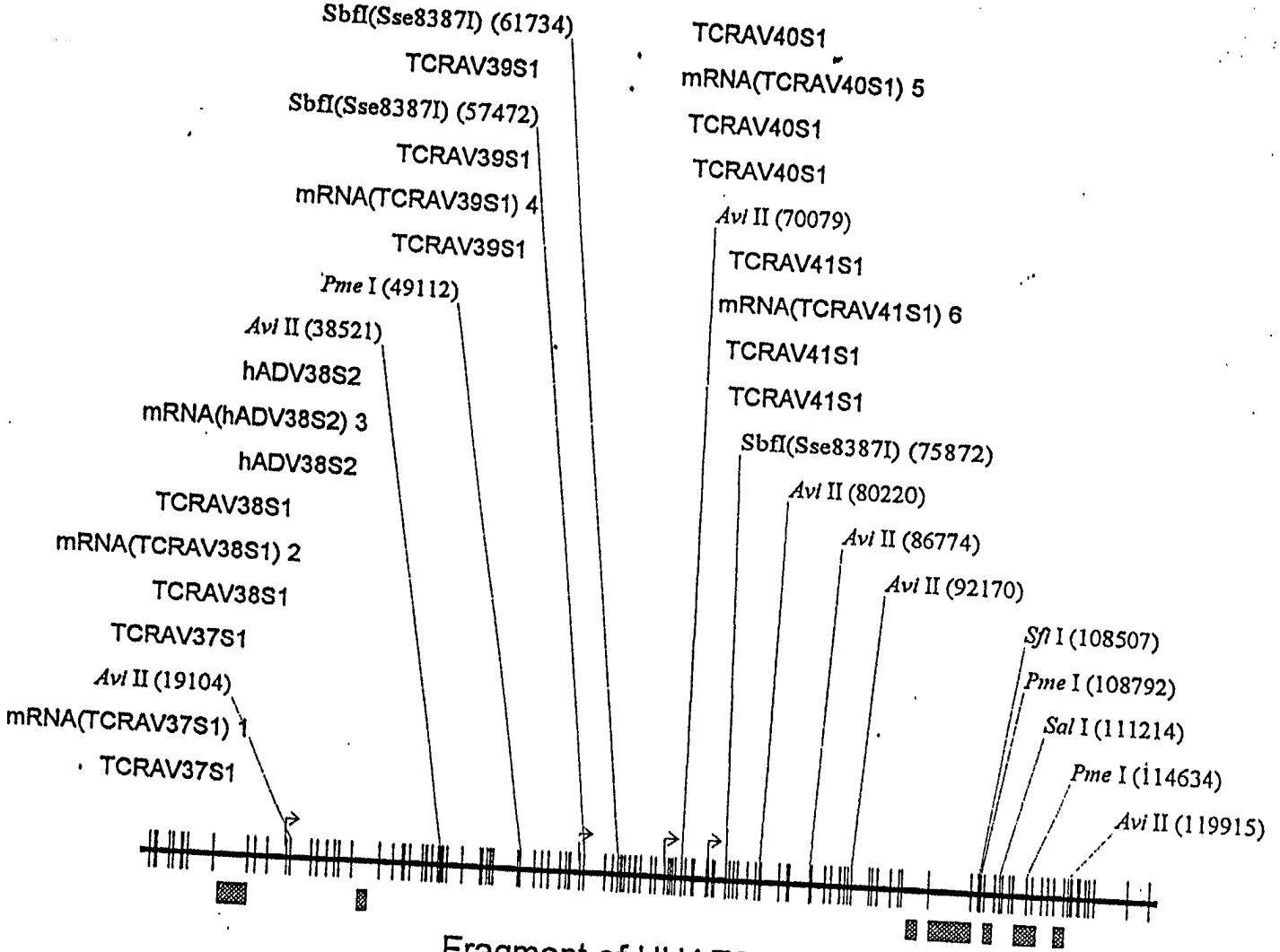
TOGTZT'8h9h200T



Fragment of HUAE000660
134892 bp (molecule 251124 bp)

Fig 4d

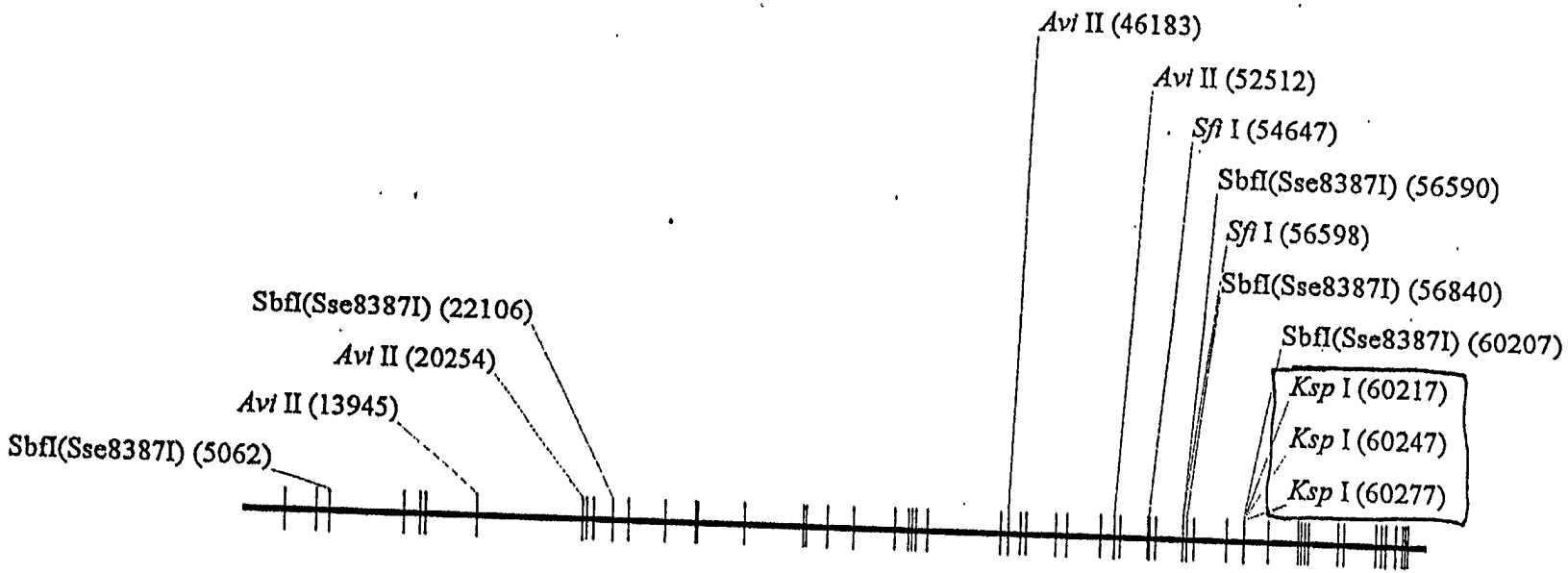
T06T2T 6h9h2001



Fragment of HUAE000661
131332 bp (molecule 247877 bp)

Fig 4e

T06727 8h9h2007

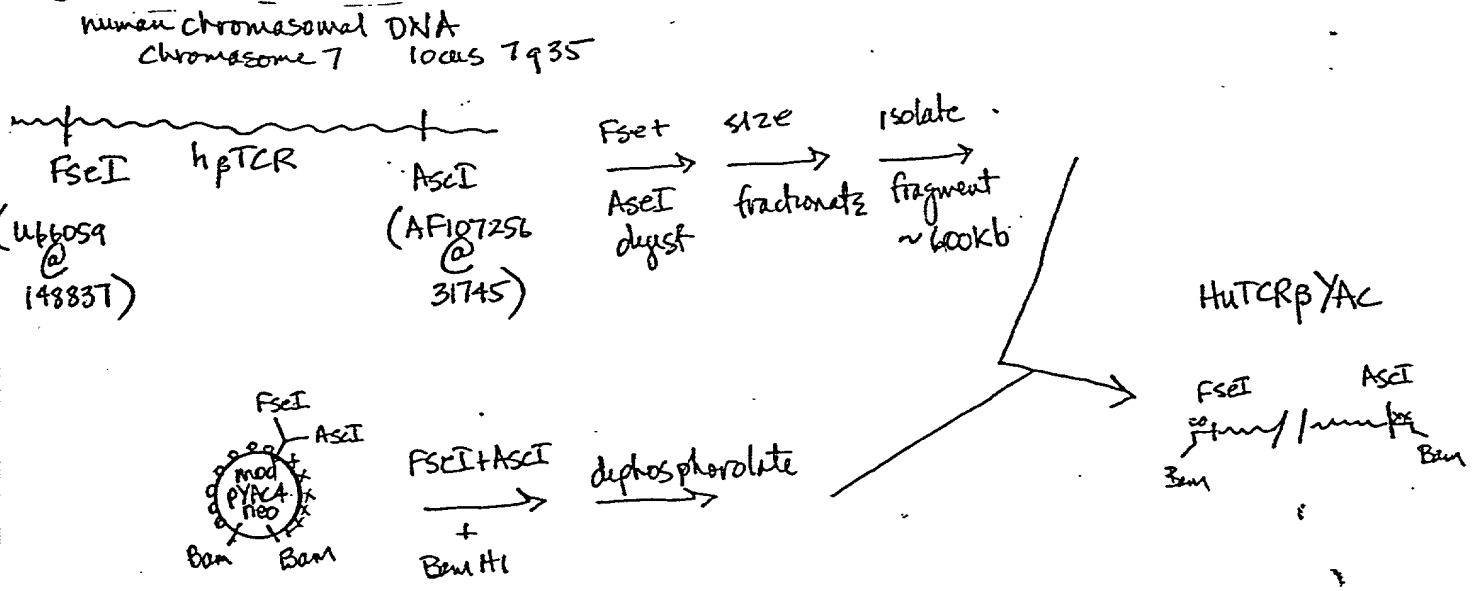


HUAE000662

71153 bp

Fig 4E

Figure 5 overview



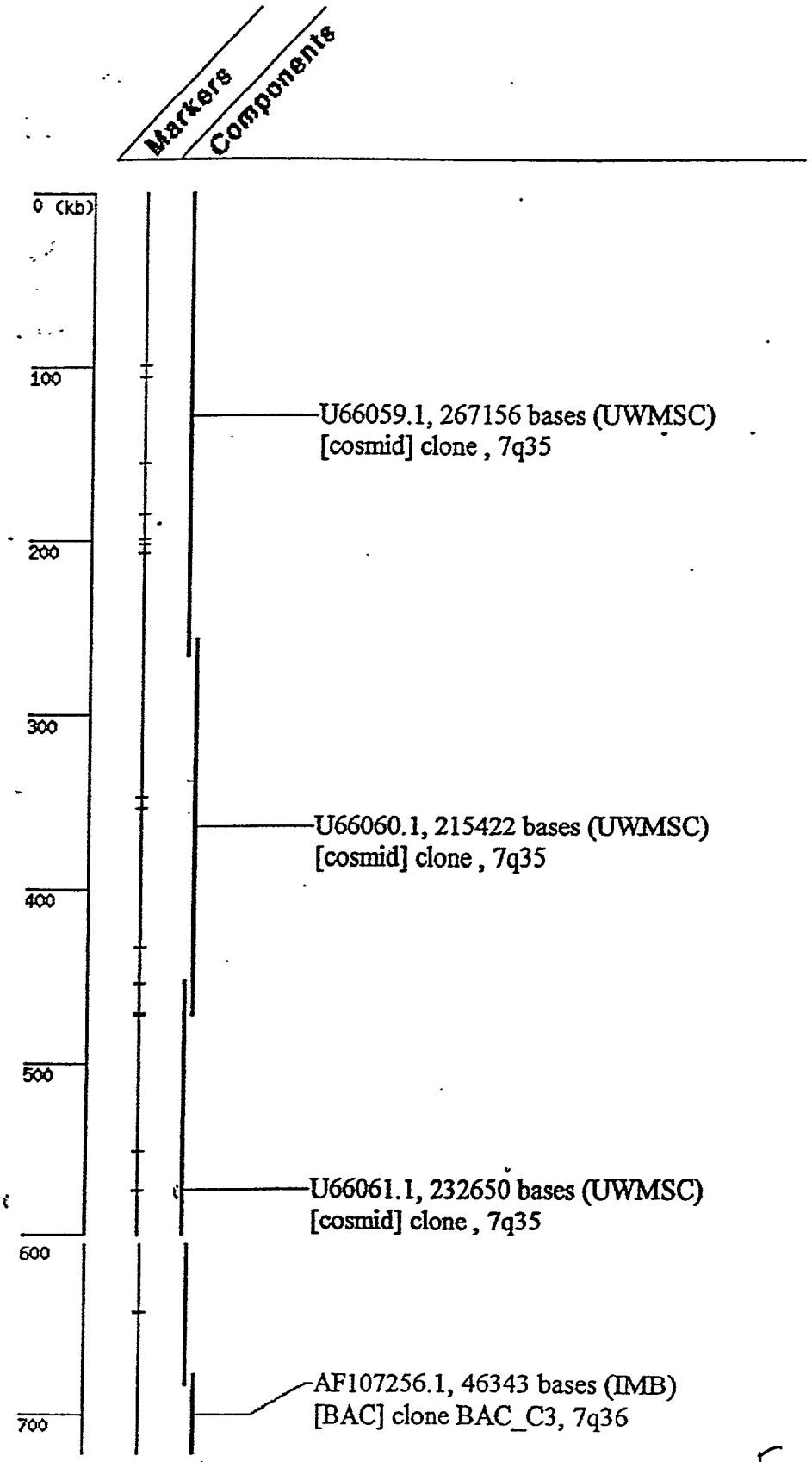
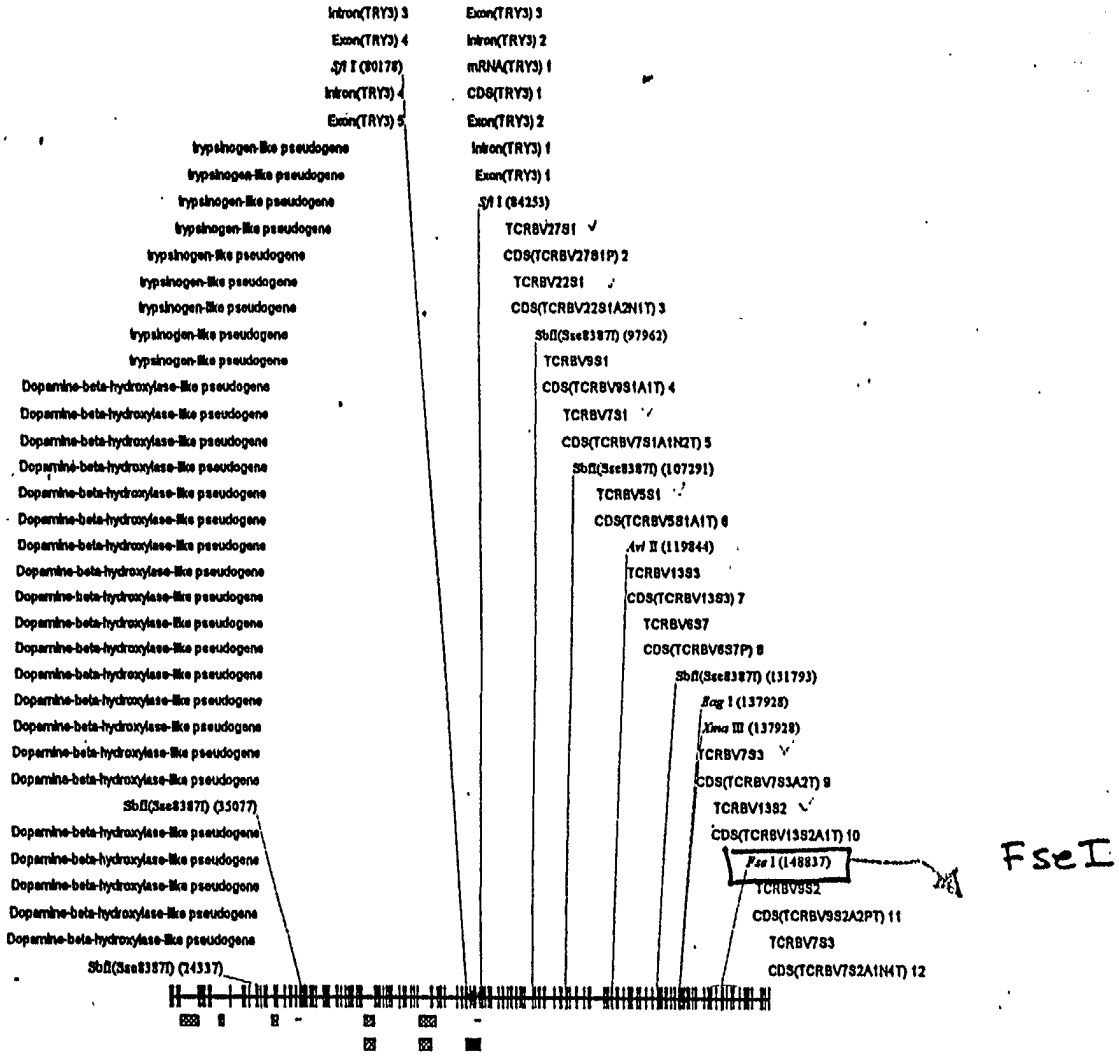

Markers
Components
0 (kb)
100
200
300
400
500
600
700
U66059.1, 267156 bases (UWMSC)
[cosmid] clone , 7q35
U66060.1, 215422 bases (UWMSC)
[cosmid] clone , 7q35
U66061.1, 232650 bases (UWMSC)
[cosmid] clone , 7q35
AF107256.1, 46343 bases (IMB)
[BAC] clone BAC_C3, 7q36

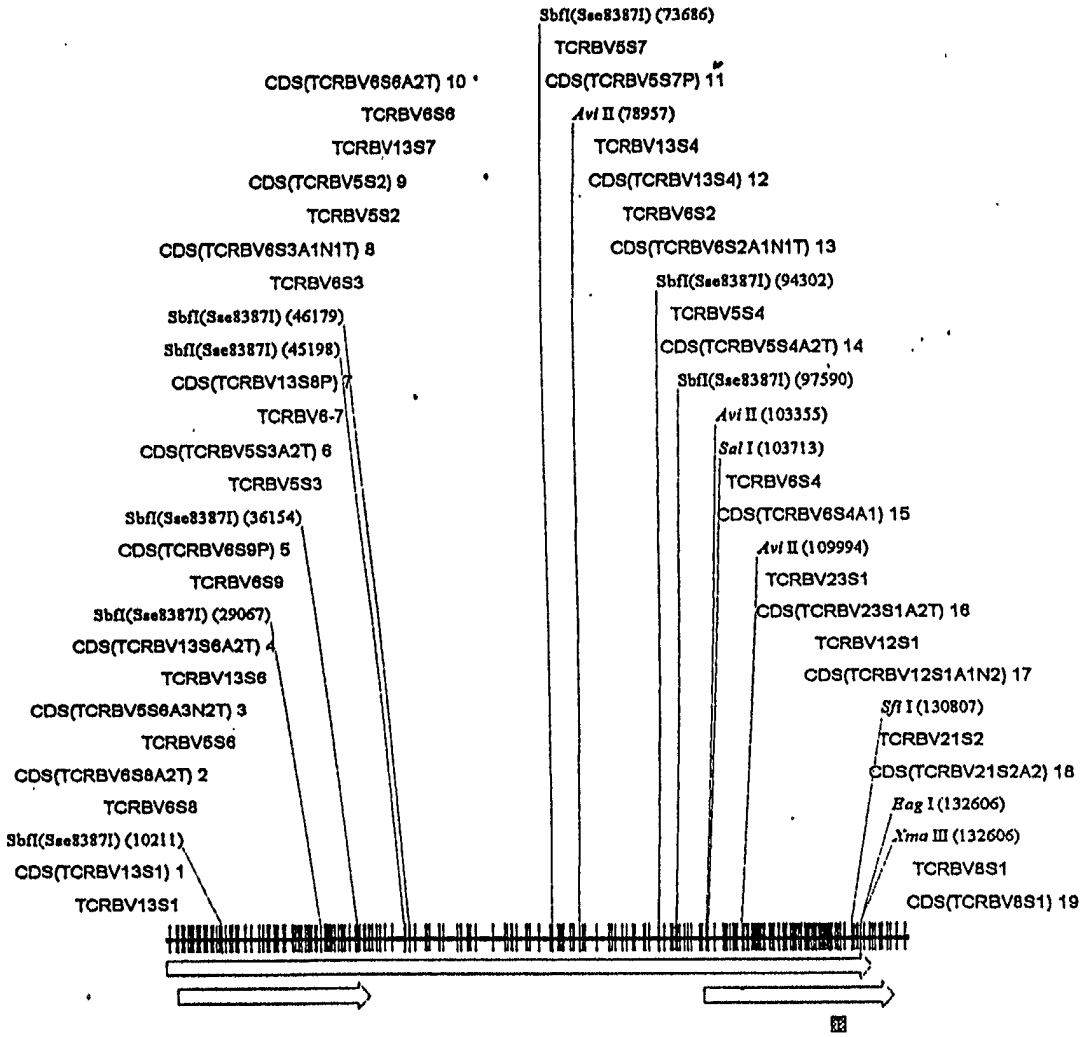
Fig 5A

TOSTAT" 8h9h200T



Fragment of U66059
161934 bp (molecule 267156 bp)

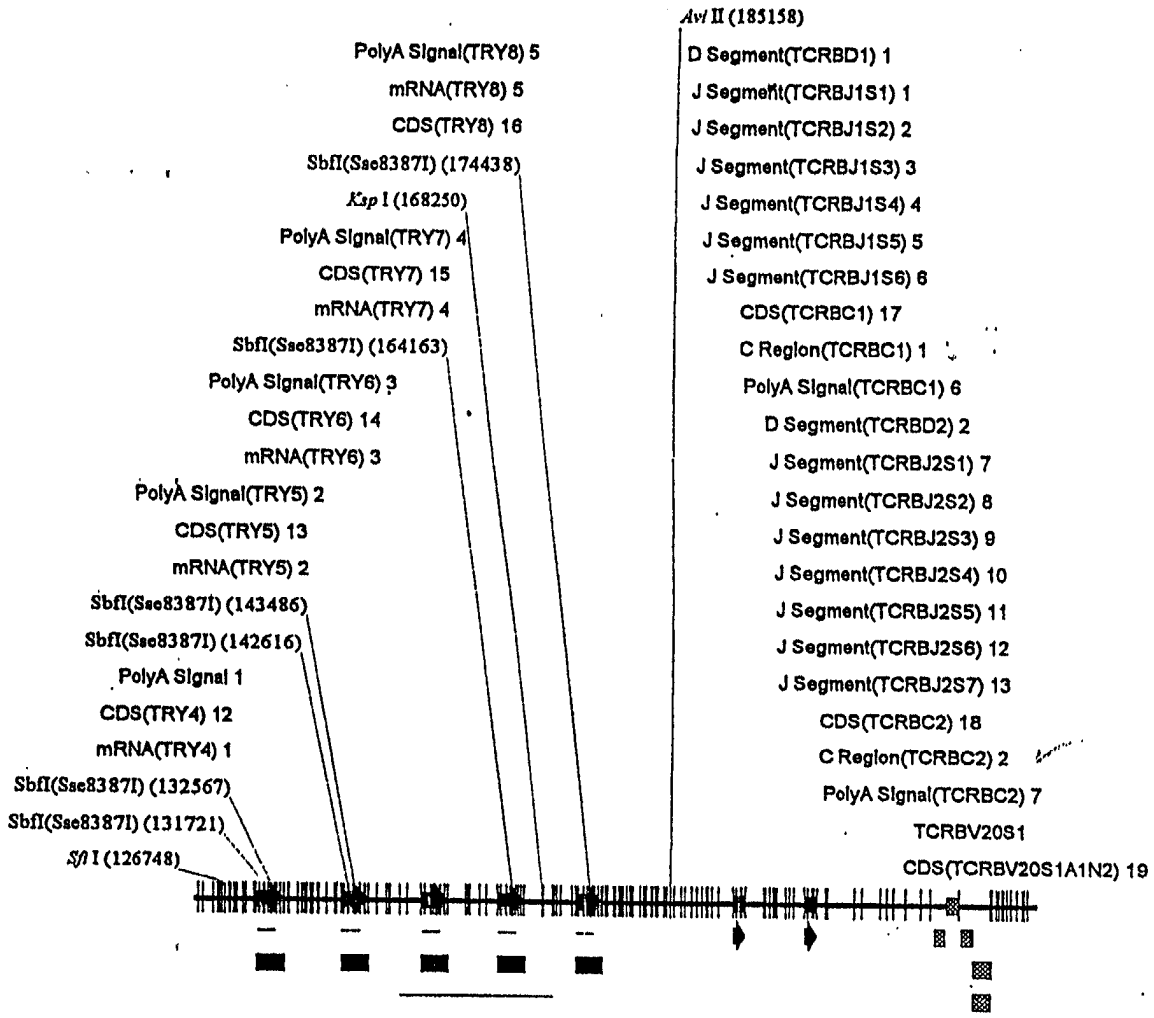
Fig 5b



Fragment of U66060
141491 bp (molecule 215422 bp)

Fig 5 C

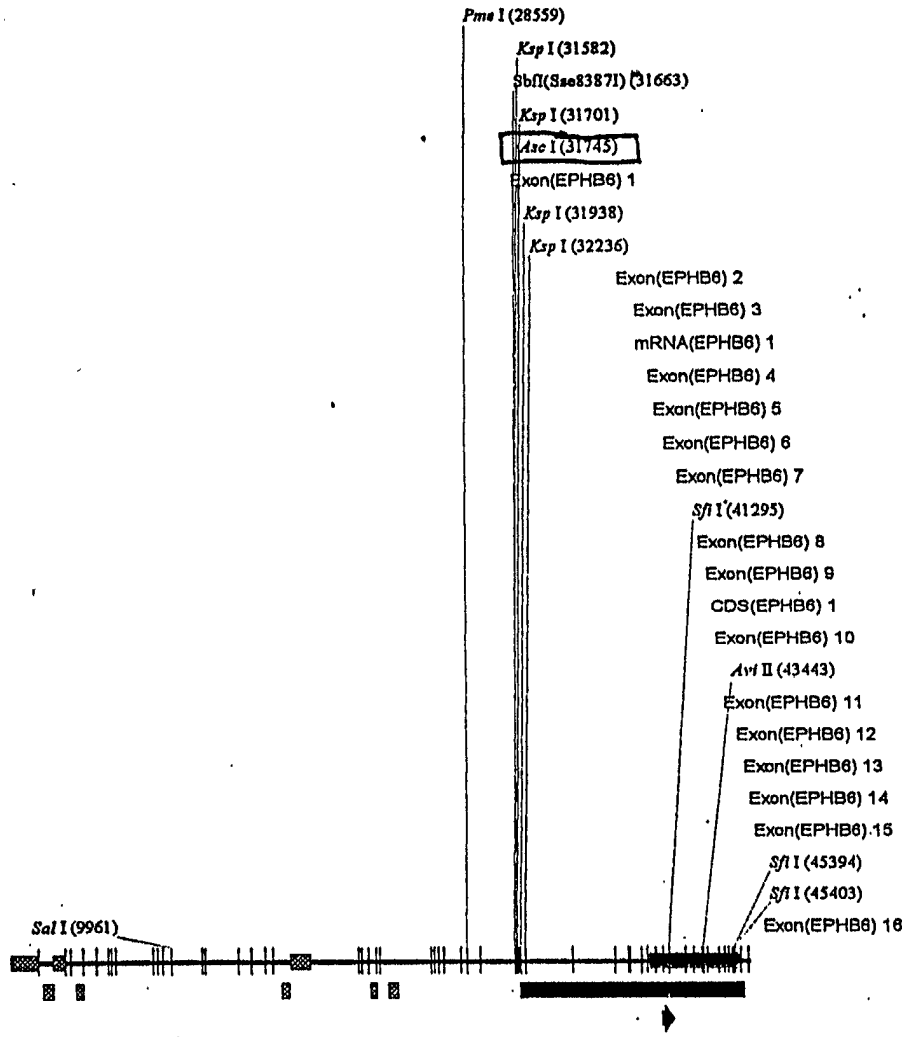
TOGTET 8h9h2007



Fragment of U66061
109812 bp (molecule 232650 bp)

Fig 5d

T06T2T" 8n9h200T



AF107266
46343 bp

Fig 5e

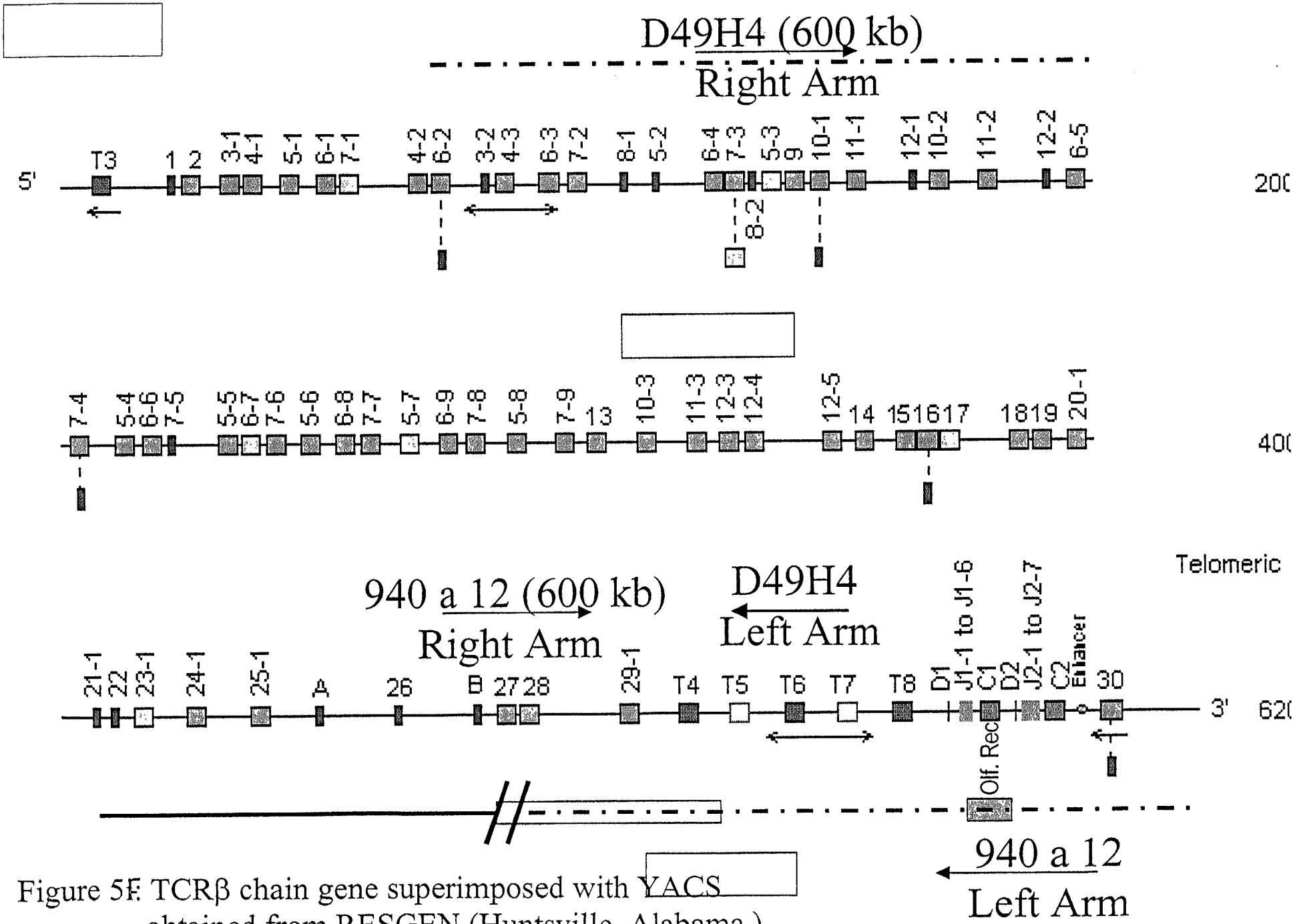
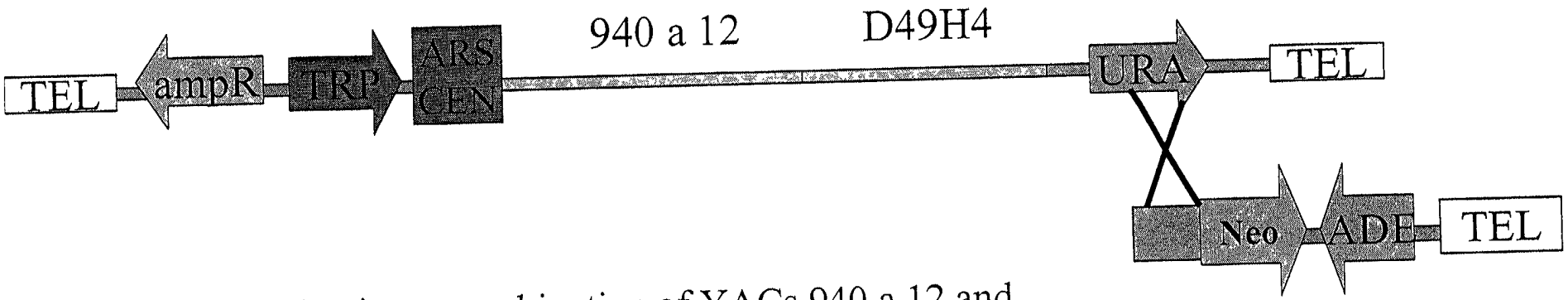


Figure 5F. TCRβ chain gene superimposed with YACS obtained from RESGEN (Huntsville, Alabama)

TOSTET 8/9/2007

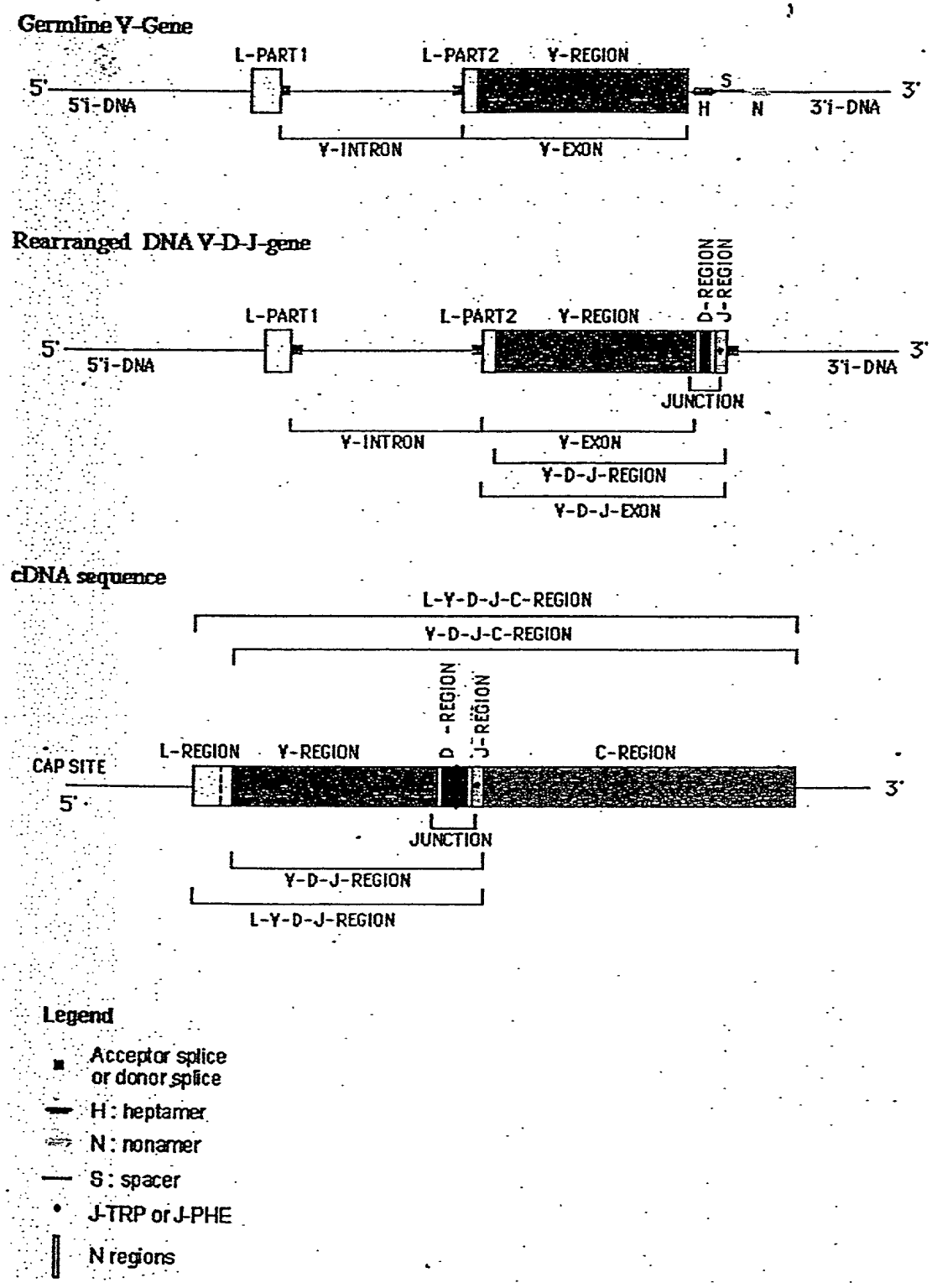
huTCRβ YAC



Modification vector

Figure 5G: Following recombination of YACs 940 a 12 and D49H4 to create the HuTCRβ YAC, the right arm of the construct can be retrofitted with vectors containing regulatory sequences and/or mammalian selection cassettes.

FIG 6


Germline V-Gene
L-PART1
L-PART2
V-REGION
5'
5'i-DNA
S
H
N
3'i-DNA
3'
V-INTRON
V-EXON
Rearranged DNA V-D-J-gene
D-REGION
J-REGION
L-PART1
L-PART2
V-REGION
5'
5'i-DNA
3'i-DNA
3'
JUNCTION
V-INTRON
V-EXON
V-D-J-REGION
V-D-J-EXON
cDNA sequence
L-V-D-J-C-REGION
V-D-J-C-REGION
D-REGION
J-REGION
L-REGION
V-REGION
C-REGION
CAP SITE
5'
3'
JUNCTION
V-D-J-REGION
L-V-D-J-REGION
Legend
Acceptor splice or donor splice
H : heptamer
N : nonamer
S : spacer
J-TRP or J-PHE
N regions